Available online at www.sciencedirect.com
ScienceDirect
Journal of Forensic and Legal Medicine 15 (2008) 27–31
JOURNAL OF FORENSIC AND LEGAL MEDICINE
www.elsevier.com/jflm

Short Report

STR typing of archival Bouin's fluid-fixed paraffin-embedded tissue using new sensitive redesigned primers for three STR loci (CSF1P0, D8S1179 and D13S317)

Stefania Turrina PhD, Renzo Atzei MD, Giulia Filippini MD, Domenico De Leo MD *

Department of Medicine and Public Health, Institute of Legal Medicine, Forensic Genetic Laboratory, University of Verona, 37134 Verona, Italy

Received 18 October 2005; received in revised form 26 October 2006; accepted 30 October 2006
Available online 18 January 2007

Abstract

Three new mini-STR primer sets are suggested for three conventional STRs, CSF1P0, D8S1179 and D13S317, included in multiplex PCR kits commercially available and commonly used for DNA typing in forensic applications. The primer pairs for the three loci were redesigned in order to reduce or eliminate the flanking regions of the polymorphism obtaining amplification products, which have dimensions less than 120 bp in size. A comparison of results for typing carried out with the newly designed primers on DNA extracted from 100 blood samples provided by healthy donors, previously typed with conventional STRs, showed no genotype difference underlining their precision and reproducibility. The forensic usefulness of the new mini-STR primers was evaluated on highly degraded DNA from casework samples (e.g. archival post-mortem Bouin's fluid-fixed paraffin-embedded tissue specimens) for which commercial STR kit had proven inefficient.

Keywords: STRs; CSF1P0; D8S1179; D13S317; Degraded DNA; Bouin's fluid-fixed paraffin-embedded tissue

1. Introduction

Paternity testing is generally ascertained using blood samples or other biological material obtained from the subjects involved in the analysis. Archival post-mortem or biopsy paraffin-embedded tissues are potential samples for DNA extraction for subsequent genetic testing for forensic applications. However, the DNA extracted from these samples is often disposable in low amount (depending on the nature and quantity of the tissue included) and degraded to various extents due to the fixing and inclusion conditions (type and time of fixing, time and storage conditions). Therefore, the DNA extracted from these samples may be characterized by low copy number where brief sequences of the degraded target DNA predominate, generally smaller than the STRs conventionally used in the PCR.[1] This negatively impacts on the amplification process leading to ambiguous results (artificial formations such as allele drop-out) or failure of the PCR process.

Previous studies showed that DNA artificially degraded leads to relatively stable fragment lengths up to 200 bp, that when submitted to PCR amplification using STR multiplex kits, only smaller STR are amplified, resulting in partial STR profiles.[2,3]

The Authors of this study have noted that from archival post-mortem Bouin's fluid-fixed paraffin-embedded tissue specimens stored at room temperature for more than 10 years, it is obtained highly degraded DNA with no significant number of intact DNA fragments greater than 120 bp. This it was inferable from the failure of the processes of amplification with AmpFlSTR Identifiler® PCR Amplification (Applied Biosystems, Foster City, CA) that did not provide any genetic profile, also when the number of

* Corresponding author. Tel.: +39 045 8074942; fax: +39 045 505259.
E-mail address: deleodom@tin.it (D.D. Leo).

1353-1131/$ - see front matter © 2006 Elsevier Ltd and FFLM. All rights reserved.
doi:10.1016/j.jcfm.2006.10.012

PCR cycles was increased from 28 to 34, with the only except for amelogenin marker, which is known to have dimensions of 106–112 bp.

An approach to trying to recover information from degraded DNA samples is to reduce amplicon lengths of PCR products by moving primers in as close as possible to the STR repeat region.[4–9]

The need to obtain a genetic profile, although limited to a few STRs by the low amount of highly degraded DNA extracted from included tissue, has led the authors to convert three conventional STRs into Mini-STRs by re-engineering, so that the primers are built close to the repeat region as recommended by EDNAP and ENFSI groups.[10]

In this case the attention was focused on STR loci, characterized by a relatively low number of repetitive units (<20), so it is possible to obtain fragment lengths less than 120 bp. For that reason, we decided to use CSF1P0, D8S1179 and D13S317 that are present in many commercial STR multiplex amplification kits (e.g. AmpFlSTR Identifiler® PCR Amplification, AB) and that are included in the standard STR loci of nationwide DNA intelligence databases in various countries.[11]

2. Materials and methods

DNA was extracted from 100 fresh blood samples provided by healthy donors (previously typed with AmpFlSTR Idenfiler® PCR Amplification) using GenomicPrep Blood DNA Isolation Kit (Ge Healthcare Bio-Sciences, Uppsala, Sweden).

DNA was extracted from five archival Bouin's fluid-fixed paraffin-embedded tissue specimens (stored at room temperature, since 1990) following manual microdissection of 10 μm thick unstained sections. The microdissection tissue fragments were de-waxed through further xylene and ethanol washes. After air drying, the tissue pellets were digested using DNA IQ™ system (Promega, Madison, WI, USA) following the manufacturer's instructions. DNA samples were measured spectrophotometrically to determine quantity and quality. Cell line K562 (Promega) was serially diluted from a concentration of 1 ng to 30 pg for sensitivity and peak imbalance study.

The three new mini-STRs were amplified in singleplex and their primer sequences are given in Table 1. The amplification conditions were the same for the three STRs.

Each PCR reaction was carried out in a total volume of 25 μL containing 10 μL DNA, 1× PCR Buffer II, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.4 μM of each primers and 1.5 units of AmpliTaq DNA polymerase (5 U/μL) (AB, Foster City, CA, USA). The primers were synthesised by Tib Molbiol (Genoa, Italy), and the forward primer from each primer set was labelled at the 5′-end with the fluorescent dye Cy5. The three STR loci were amplified at the same conditions: 95 °C for 2 min, followed by 34 cycles at 95 °C for 45 s, 54 °C for 45 s, 72 °C for 30 s, and a final extension step at 72 °C for 10 min. All reactions, together with positive and negative control samples, were performed in a GeneAmp® PCR System 9700 Thermal Cycler (AB, Foster City, CA, USA).

Samples were set up for electrophoresis by combining 5 μL of each PCR product to 3 μL of loading dye and 2 μL 50–500 bp Size Standard (Amersham Biosciences). The same fluorescently labeled size standard (50–500 bp) was also used as an external standard to compensate for any mobility shift between the lanes. After denaturation at 95 °C for 3 min and subsequently chilled on an ice block for 3 min, the samples were resolved through electrophoresis in a 6% w/v polyacrylamide gel with a 19:1 ratio of acrylamide/bisacrylamide (Ready Mix Gel ALF grade, Amersham Biosciences, Buckinghamshire, England). Electrophoresis was carried out by Automatic Laser Fluorescent (ALFexpress) DNA sequencer (Pharmacia-Biotech, Uppsala, Sweden) at 1450 V, 38 mA, 45 W and 48 °C with laser power at 3 mW for 100 min. Raw data were assigned fragment sizes in base pairs with reference to the internal standard, using analysis Fragment Manager Software V 1.2 run under OS/2. Genotype assignment was done by comparison with sequenced allelic ladders and allele designation following the recommendations of the DNA Commission of the ISFH.[12]

Furthermore, DNA extract from five archival Bouin's fluid-fixed paraffin-embedded tissue was amplified with AmpFlSTR Identifiler® PCR Amplification in accordance with the instructions provided by the manufacturer (Applied Biosystems) and the numbers of PCR cycles were

Table 1
Primer sequences and product sizes used in this study

Locus	Mini-STR primers (5′ → 3′)	Allele range[a]	Mini-STR size (bp)	STR kit product size (bp)	Size reduction (bp)
CSF1P0	5′-CATAGATAGAAGATAGATAG-3′ 5′-CCTGTTCTAAGTACTTCC-3′	6–16	66–106	306–346 (Identifiler)	240
D8S1179	5′-GTATTTCATGTGTACATTCG-3′ 5′-GATTATTTTCACTGTGGGG-3′	7–19	71–119	124–172 (Identifiler)	53
D13S317	5′-CTATCTGTATTTACAAATAC-3′ 5′-CAGAAAGATAGATAGATG-3′	5–16	64–108	205–249 (Identifiler)	141

[a] As reported in the literature; see STR Fact Sheets in STRBase (http://ibm4.carb.nist.gov:880/dna/home.htm).

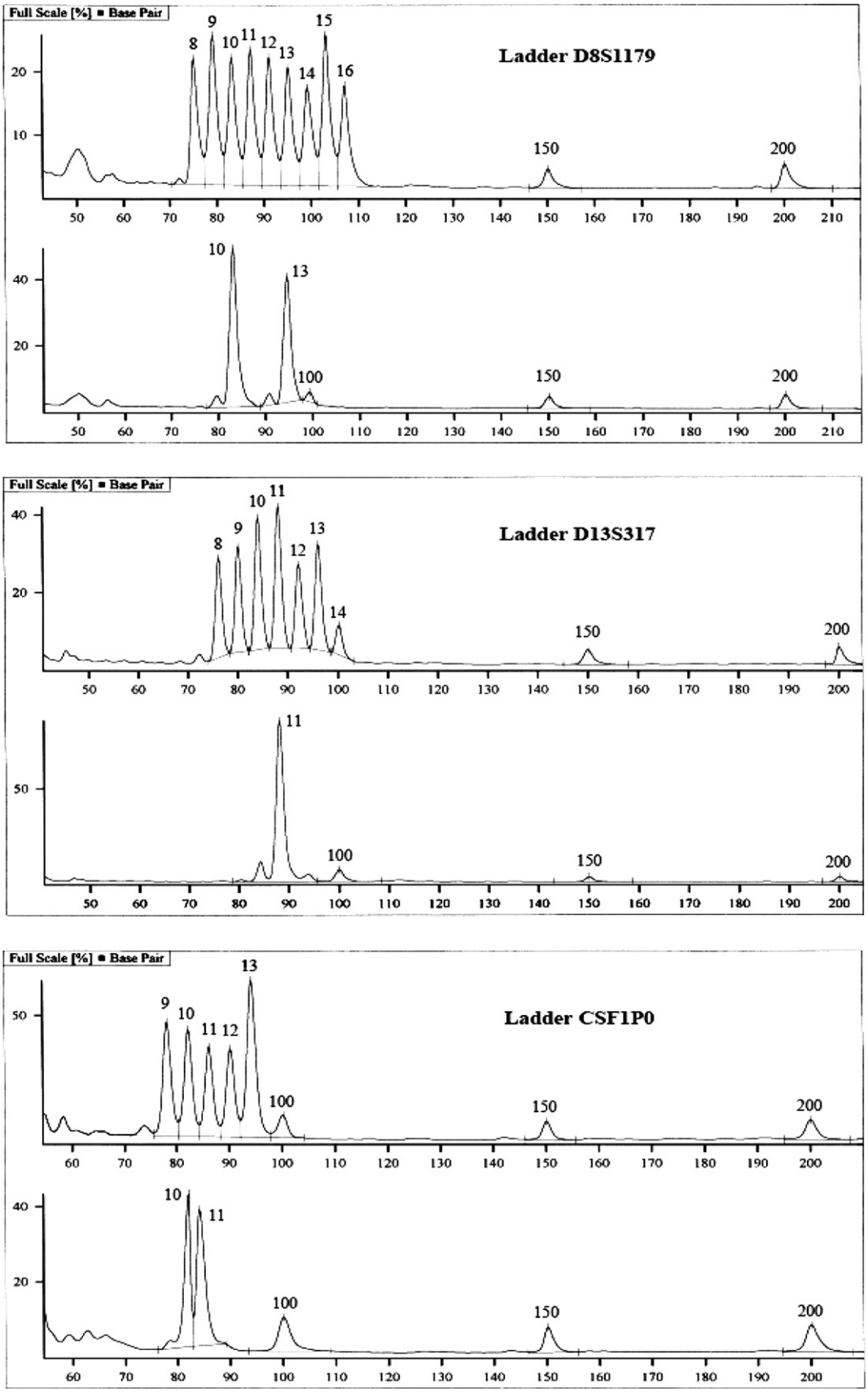

Fig. 1. Electropherogram of the amplification of a highly degraded DNA from archival Bouin's fluid-fixed paraffin-embedded tissue specimen with the three mini-STRs.

increased from 28 to 34 to be more sensitive for the samples typing.

3. Results and discussion

Mini-STRs can be obtained by positioning the primer sets as close as possible to the ends of repeat regions. Recently, a core set of smaller STRs have been developed for utilization in forensic casework,[13] but a few of these loci have not been made into smaller amplicons. We observed that not all conventional STRs included in multiplex amplification kits can be transformed into very small STRs less than 120 bp in size, but only those that are characterized by a limited number of repetitive units, no more than 20 units, in other word only STRs that have short allele ranges.

The new mini-STR primer sets developed by the authors have allowed to obtain PCR products with small length less than 120 bp in size, so that for the CSF1PO, with allele range of 6–16, there has been a shift from 306–346 bp (AmpFlSTR Identifiler, ABI) to 66–106 bp; for the D8S1179, with allele range of 7–19, the shift has been from 124–172 bp (AmpFlSTR Identifiler, ABI) to 71–119 bp; and for the D13S317, with allele range of 5–16, the shift has been from 205–249 bp (AmpFlSTR Identifiler, ABI) to 64–108 bp (as shown in Table 1).

A sensitivity study has shown that the PCR process could also be carried out at a concentration of 30 pg of genomic DNA (K562, Promega) providing reproducible results (data not shown), even if at this concentration heterozygote peak height imbalance was observed. The comparison of typing results between the three new mini-STRs and conventional STRs (AmpFlSTR Identifiler, ABI) on a sample of 100 healthy donors showed no genotype differences with good balance between alleles, no double peaks due to +A/−A and stutter products higher less than 15% of the main peak; confirming that changes in the dimensions of the STRs do not influence the profile.

The application of three new Mini-STRs primers in forensic cases characterized by highly degraded DNA has proven efficient in the amplification process in which conventional STRs failed to provide any profiles. In particular, they were very useful in a case of paternity testing involving DNA extracted from archival tissue included in paraffin and embedded with Bouin's fluid.

It is widely demonstrated that fixing and inclusion of the tissue (type of embedding agent and time of inclusion) can influence the quality and quantity of DNA extracted. One of the factors that play an important role in the various alteration processes of the structure of the DNA certainly includes the type of embedding agent, which in most cases is formalin used alone or in association with other fixing agents. The action of formalin leads to DNA strand breakage and degradation, DNA depurinization and DNA sequestration in protein–DNA complex.[14] Bouin's fluid is composed of a saturated aqueous solution of formalin, glacial acetic acid and picric acid, so that the degradation of DNA by formalin is increased by the interaction with the two acids. Several authors have reported the impossibility of typing DNA extracted from embedded blocks due to acid environment of Bouin's fixative that causes strong DNA degradation.[15–17]

The DNA extracted from five archival Bouin's fluid-fixed paraffin-embedded tissue specimens showed a higher degree of degradation so that PCR amplification with commercially available multiplex STR kit was not possible, except for amelogenin (106–112 bp). In fact, only amplicons less than 120 bp in size could be successfully amplified. In this case the three new mini-STRs provided a genetic profile (see Fig. 1), increased to six STRs using three microsatellites (TH01, TPOX, FES) recommended by Hellmann et al., also with size below 120 bp.[8] For the five archival Bouin's fluid-fixed paraffin-embedded tissue samples, the increased typing success can be attributed to the combined use of the new mini-STRs and the singleplex amplification format, but this does not exclude the use of the mini-STRs in multiplex PCR. In this case it was not possible to amplify the three Mini-STRs in a multiplex format because the primers were labelled with the same fluorescent dye (Cy5).

In conclusion, the use of Mini-STR primer sets may be a good alternative or additional tool when conventional STRs are not able to provide useful information from highly degraded DNA samples.

Acknowledgement

This work was supported by a grant from " Donazione Miria Cherubini e Amleto Loro".

References

1. Romero RL, Juston AC, Ballantyne J, Henry BE. The applicability of formalin-fixed and formalin fixed paraffin embedded tissues in forensic DNA analysis. *J Forensic Sci* 1997;**42**(4):708–14.
2. Schneider PM, Bender K, Mayr WR, et al. STR analysis of artificially degraded DNA – results of a collaborative European exercise. *Forensic Sci Int* 2004;**139**:123–34.
3. Bender K, Farfan MJ, Schneider PM. Preparation of degraded human DNA under controlled conditions. *Forensic Sci Int* 2004;**139**:135–40.
4. Yoshida K, Sekiguchi K, Kasai K, Sato H, Seta S, Sensabaugh GF. Evaluation of new primers for CSF1P0. *Int J Legal Med* 1997;**110**:36–8.
5. Ricci U, Giovannucci Uzielli ML, Klintschar M. Modified primers for D12S391 and a modified silver staining technique. *Int J Legal Med* 1999;**112**:342–4.
6. Wiegand P, Kleiber M. Less is more – length reduction of STR amplicons using redesigned primers. *Int J Legal Med* 2001;**114**:285–7.
7. Grubwieser P, Muhlmann R, Parson W. New sensitive amplification primers for the STR locus D2S1338 for degraded casework DNA. *Int J Legal Med* 2003;**117**:185–8.
8. Hellmann A, Rohleder U, Schmitter H, Wittig M. STR typing of human telogen hairs – a new approach. *Int J Legal Med* 2001;**114**:269–73.
9. Tsukada K, Takayanagi K, Asamura H, Ota M, Fukushima H. Multiplex short tandem repeat typing in degraded samples using newly designed primers for the TH01, TPOX, CSF1P0 and vWA loci. *Legal Med* 2002;**4**:239–45.

10. Gill P, Fereday L, Morling N, Schneider PM. The evolution of DNA databases – recommendations for new European STR loci. *Forensic Sci Int* 2006;**156**:242–4.
11. Schneider PM, Martin PD. Criminal DNA database: the European situation. *Forensic Sci Int* 2001;**119**:232–8.
12. Bär R, Brinkmann B, Budwole B, Carracedo A, Gill P, Lincoln P, et al. DNA recommendations: further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems. *Int J Legal Med* 1997;**110**:175–6.
13. Butler JM, Shen Y, McCord BR. The development of reduced size STR amplicons as tools for analysis of degraded DNA. *J Forensic Sci* 2003;**48**(5):1054–64.
14. Farrand K, Jovanovic L, Delahunt B, Mclver B, Hay ID, Eberhardt NL, et al. Loss of heterozygosity studies revisited. *J Mol Diagn* 2002;**4**:150–8.
15. Greer CE, Peterson SL, Kiviat NB, et al. PCR amplification from paraffin-embedded tissues. Effects of fixative and fixation time. *Am J Clin Pathol* 1991;**95**:117–24.
16. Gall K, Pavelic J, Jadro-Santel D, et al. DNA amplification by polymerase chain reaction from brain tissues embedded in paraffin. *Int J Exp Pathol* 1993;**74**:333–7.
17. Hunt JL, Finkelstein D. Microdissection techniques for molecular testing in surgical pathology. *Arch Pathol Lab Med* 2004;**128**: 1372–8.